Molecular Disease and Evolution

by

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The universe is made up of matter and radiant energy. The human body is made up of molecules - molecules of all sorts; little molecules, such as the water molecule, consisting of only three atoms - a very important molecule, which is present in larger numbers than any other in the human body; larger molecules, of medium size, such as those that constitute the vitamins; and many very large molecules, protein molecules, polysaccharide molecules, nucleic acid molecules.

I believe that it is likely that a human being manufactures 50,000 or 100,000 different kinds of protein molecules. A representative protein molecule, such as hemoglobin, is built of about 10,000 atoms. It has a well defined structure; for most of the protein molecules not a single atom is out of place.

The protein molecules of different kinds are manufactured by genes, which are themselves molecules of deoxyribonucleic acid. Each one of us inherits half of his complement of genes, approximately 50,000 from his father, and the other half, approximately 50,000, from his mother. It is these molecules, 100,000 molecules of DNA, that make the human being what he is, that confer his characters upon him.

These are the most important molecules in the world. The pool of human germ plasm is a precious heritage of the human race.

A few years ago it was discovered that some diseases are molecular diseases, diseases of protein molecules. A gene, a molecule of deoxyribonucleic acid, may be damaged by cosmic radiation or some other mutagenic agent in such a way that a few atoms are out of place. This gene then duplicates itself in its new, mutated, form. Moreover, when it serves its other function, the function other than self-duplication, it determines the nature of a protein molecule, which it has the responsibility of manufacturing. A mutated gene produces an altered protein molecule, with a few atoms different from the corresponding normal protein molecule.

Molecular disease is closely connected with evolution. The appearance of the concept of good and evil that was interpreted by Man as his painful expulsion from Paradise probably was a molecular disease that turned out to be evolution.

Among the molecular diseases there are many that involve enzymes. For example, the disease phenylketonuria, which is responsible for 1% of the institutionalized mentally defective individuals in the United States, is a simple molecular disease that is reasonably well understood. One person in eighty has an

abnormal gene that is called the gene for phenylketonuria. A normal person has two genes that manufacture, independently of one another, an enzyme is the liver that catalyzes the oxidation of phenylalanine to tyrosine. This is a mechanism for converting part of the phenylalanine in our food, which is present in excess over our need, into another amino acid, tyrosine, which is then used in various ways in the human body. One person in eighty has one normal gene, which manufactures this enzyme, and one abnormal gene (the gene for phenylketonuria), which does not manufacture the enzyme, or which manufactures an abnormal enzyme molecule that is lacking in enzyme activity.

These people, the carriers of a single gene for phenylketonuria, manufacture only 50% as much of the enzyme as normal individuals; but this 50% is enough to take care of the phenylalanine that they ingest. They are called phenylketonuric heterozygotes. They are not damaged significantly by carrying the gene in single dose.

However, when two of these heterozygotes marry one another there occurs the great lottery, the greatest of all lotteries in the world, in which the prospective child, the fertilized ovum, carries out the selection of one or the other of the pair of genes that the father has and of one or the other of the pair of genes that the mother has. On the average, a quarter of the children inherit the defective gene from the father and also the defective gene from the mother. They have the defective gene in double dose, and they manufacture none of the enzyme that catalyzes the oxidation of the phenylalanine to tyrosine. When such a homozygote eats his food, containing ordinary protein, the phenylalanine builds up in his blood stream and cerebrospinal fluid to concentrations as great as fifty times that in normal individuals. This high concentration of phenylalanine and of other substances made from it interferes with the growth and function of the brain in such a way as to cause him to be mentally defective, perhaps with an I.Q. as low as 20. In addition, the phenylketonuria genes in double dose cause him to have severe eczema and other somatic difficulties.

It has been recognized in recent years that it is possible to treat this disease, phenylketonuria. A diagnosis of the disease may be made at an age as early as one month, and the infant then may be fed a diet of protein hydrolysate from which most of the phenylalanine has been removed. Children treated in this way seem to develop in an essentially normal manner.

Many molecular diseases that have arisen in the course of evolution have been controlled in a somewhat similar manner. Human beings require many vitamins. Pellagra is an example of a vitamin deficiency disease - a molecular disease that originated through a mutation, perhaps millions of years ago, and was then cured by the heterotrophic process of eating other organisms that manufacture the vitamin. Scurvy and other avitaminoses are also diseases of this sort. It is not customary for us to admit that we have these diseases, because we treat them as a matter of habit by eating what is called a proper diet.

Organisms such as the red bread mould are able to manufacture not only all

of the vitamins, but also all of the amino acids. At some time in our evolutionary history we suffered mutations that resulted in the loss of our power to manufacture the various enzymes involved in these syntheses. Each of these mutations produced in our predecessors a disease - one disease for each vitamin that we now require, and one disease for each of the nine amino acids that are essential for man. Most of us keep these diseases under control by ingesting the proper food.

I have been especially interested in the hemoglobinopathies, which are the diseases, including sickle-cell anemia, to which the name molecular disease was first applied. I remember very well the time, some fifteen years ago, when three of my students - Dr. Harvey Itano, Dr. S. J. Singer, and Dr. I. C. Wells, carried out the crucial experiment that showed that sickle-cell anemia is a disease of the hemoglobin molecule. I had made this prediction three years earlier, and Dr. Itano had worked for three years, toward the end with Dr. Singer and Dr. Wells, to test it.

Patients with sickle-cell anemia are anemic because their red cells tend to twist out of shape. These deformed cells are then recognized by the spleen as abnormal, and are destroyed so rapidly as to make it impossible for the patient to manufacture new erythrocytes fast enough to prevent anemia from developing. Moreover, the deformed cells are sticky; they clump on to one another and clog up the capillaries in such a way as to interfere with the flow of blood and thus to cause different organs of the body to be damaged by anoxia. This disease, involving deformation of the red cell, might seem to be a classic disease of cells, as described by Rudolf Virchow. However, the fact that the cells sickle only in the venous circulation and regain their normal shape in the arterial circulation seemed to me, in 1945, to provide very strong indication that the disease is in fact a disease of the hemoglobin molecule, which is present as hemoglobin in the venous blood and as a different molecule, oxyhemoglobin, in arterial blood.

We all know that protein molecules tend to be sticky - it is hard for a protein molecule to keep from being sticky. If a solution of protein molecules, manufactured by some living organism and selected by the evolutionary process of trial and error so as not to be sticky, but to remain in solution instead of forming an insoluable coagulum, is disturbed a bit by warming, even to as low a temperature as 60°C, so that the molecules become slightly unfolded (denatured), then the characteristic property of stickiness makes itself evident; the denatured protein molecules clump onto one another, to form an insoluble coagulum of denatured protein. It need not surprise us that, although the normal hemoglobin molecules, selected by the evolutionary process, are able to remain separated from one another even in the concentrated solution (30% protein) that is inside the red cell, a change in structure resulting from a gene mutation may cause the altered hemoglobin molecule to have a sticky region on its surface, such as to make it tend to clump onto another one, which would clump onto a third one, a fourth one, and so on, to form a long rod of these molecules. These rods would then line up side by side, attracted by the Van der Waals forces of attraction, to form a sort of needle-like crystal that would grow longer and longer

until, as it became longer than the diameter of the red cell, it would twist the red cell out of shape, and would deform the red cell membrane, making it sticky and causing the red cells to get tangled up with one another in the capillaries and causing the spleen to destroy these red cells, and thus produce the manifestation of the disease. We accordingly have a molecular explanation of the manifestations of the disease, based upon the hypothesis that is a disease of the hemoglobin molecule, a molecular disease in which the abnormal molecule is manufactured by a mutated gene. We can also understand that the molecules of exihemoglobin, molecules of hemoglobin to which molecules of oxygen molecules to interfer with the Van der Waals forces of attraction and thus to prevent the sickling of the red cells in the arterial circulation.

The incidence of the gene for phenylketonuria is small enough to permit it to be explained as the result of a study state determined by the rate at which new genes for phenylketonuria are produced by mutation and the rate at which the phenylketonuria genes are removed from the pool of human germ plasm by the death without propeny of the phenylketonuria homozygotes. But the incidence of the gene for sickle-cell hemoglobin is much too great to be explained in this way. It was recognised that the sickle-cell gene must carry some advantageous character, to compensate the disadvantage of death of the sickle-cell homozygotes without propeny. The suggestion was made by Dr. Russell Brain that the heterozygotes, carrying one sickle-cell gene, are protected against malaria - he had noticed that there is a higher incidence of sickling in villages in Africa where malaria is endemic than in other villages, where malaria is not endemic. Dr. Anthony Allison, of Oxford, then carried out an experiment that provided good evidence that the sickle-cell heterozygotes are protected against malignant subtertian malaria (Plasmodium falciparum). We can accordingly understand why the sickle-cell gene spread in the Africian population. A heterozygote, carrying a sickle-cell gene newly formed through mutation, was protected against malaria. Half of his children inherited the sickle-cell gene, and, because of their protection against malaria, they helped in rapidly spreading the gene through the population. Finally, the incidence of the gene approached the steady-state value. In marriages between heterozygotes, who would then make up a large fraction of the population, one quarter of the children would inherit two normal genes for hemoglobin, and would, in large part, die of malaria; one quarter would inherit two sickle-cell genes,

and would die of sickle-cell anemia; but one half would be heterozygotes, like their parents, and would be protected against malaria and would not have the disease sickle-cell anemia. This process gives a yield of only fifty percent in children, but only recently has the yield of fifty percent been thought to be unsatisfactory.

The next step in the process should be a mutation that would manufacture a kind of hemoglobin such that in the homozygous state it would provide protection against malaria and would not produce a disease such as sickle-cell anemia. This newly mutated gene could spread rapidly through the population, provided that the double heterozygotes, in the new gene and in the sickle-cell gene, were also protected against malaria and did not have a serious disease. It seems not unlikely that another known form of abnormal hemoglobin, hemoglobin C, represents a step in this direction.

Since the discovery of sickle-cell anemia hemoglobin 14 years ago some scores of other abnormal human hemoglobins have been discovered. These abnormal hemoglobins are associated with many different diseases.

The nature of the difference between sickle-cell-anemia hemoglobin (hemoglobin S) and normal adult human hemoglobin (hemoglobin A) has now been discovered, through the efforts principally of Vernon M. Ingram and his collaborators. Immediately after the discovery of hemoglobin S, Dr. Walter A. Schroeder and his associates in the California Institute of Technology made an amino-acid analysis of hemoglobin S and hemoglobin A. They were able to report that the amino-acid composition of the two hemoglobins is closely similar, with no amino-acid represented by a difference of more than two residues. Ingram then developed a new and powerful way of investigating the structure of hemoglobin molecules, which he called the fingerprint method - it is also called the peptide-pattern method. The sample of hemoglobin is split into peptides by the proteolytic action of an enzyme, such as trypsin. About twenty-six peptides, containing on the average about twelve amino-acid residues each, are obtained in the mixture produced in this way. The mixture is then separated into the constitutent peptides by a two-dimensional process carried out on a sheet of filter paper. The separation on the basis of mobility in an electric field is carried out along the horizontal axis of the sheet of filter paper, and then separation by the chromatographic method, involving a flowing solvent, is carried out in the vertical direction.

In this way Ingram was able to show that hemoglobin S differs from hemoglobin A only in the replacement of a single amino-acid residue in one-half of the hemoglobin molecule by the residue of another amino acid.

Schroeder and his associates in Pasadena found that hemoglobin molecules usually consist of four polypeptide chains, two of one kind and two of another kind. The normal adult human hemoglobin molecule contains two alpha chains, which have the sequence val-leu-ser-pro-ala... (total 141 residues), measured from the free-amino end, and two beta chains, which have the sequence val-his-leu-thr-pro-glu-... (total 146 residues). Ingram and Schroeder found that the alpha chains of hemoglobin S are the same as those of hemoglobin A, but the beta chains are different: the beta chain of hemoglobin S has valine in the sixth position, in place of glutamate; the other 145 residues are the same.

The amino-acids sequence has been determined for many other abnormal hemoglobins. For every one of those studied so far, the mutation involves only a single amino-acid residue. Thus, for hemoglobin C there is lysine in the sixth residue of the beta chain, in place of the glutamate of hemoglobin A or the valine of hemoglobin S. For hemoglobin E there is lysine in the twenty-sixth position, in place of glutamate.

Other abnormal hemoglobins involve an abnormality in the alpha chain, rather than the beta chain. An interesting example is hemoglobin M_{Boston}. In the alpha chain of normal adult hemoglobin the 58th residue is histidine. This residue of histidine is known to be close to the iron atom of the heme group. Histidine usually carries a positive charge, because of the attachment of a proton to the imidazole ring. In hemoglobin M_{Boston} the alpha chain has tyrosine in the 58th position, in place of histidine. Because the tyrosine residue does not carry a positive charge, we may expect that it would be easier for the iron atom of the heme group to assume an extra positive charge, leading to a ferri heme group, containing tripositive iron, in place of the normal ferro heme, containing bipositive iron. The presence of tripositive iron in hemoglobin converts it into ferrihemoglobin (also called methemoglobin); and the carriers of the gene for hemoglobin M_{Boston} do in fact have a disease, a form of methemoglobinemia.

Accordingly in this disease, as in sickle-cell anemia, the known difference in amino-acid sequence of the abnormal hemoglobin provides a reasonable explana-

tion of the manifestations of the disease produced by the molecular abnormality.

Some interesting conclusions about the process of evolution have been reached on the basis of the comparison of amino-acid sequence of hemoglobin molecules of animals of different species, carried out especially by my collaborator Dr. Emile Zuckerkandl. It has been found that the peptide pattern of hemoglobins of animals of different species can be correlated reasonably well with the generally accepted ideas about evolutionary relationships between the species. For example, the peptide patterns of gorilla hemoglobin and chimpanzee hemoglobin are almost identical with those of human hemoglobin. The peptide pattern of Rhesus monkey hemoglobin is somewhat different from that of human hemoglobin. Still greater differences from human hemoglobin are shown by the patterns of cow hemoglobin, horse hemoglobin, pig hemoglobin, and the hemoglobins of other mammals. The differences are greater still for fish hemoglobin and worm hemoglobin.

A detailed study of horse hemoglobin has shown that the alpha chains differ from those of human hemoglobin by about 18 amino-acid substitutions, as do also the beta chains of the two hemoglobins. If we accept 130,000,000 years as the time that has passed since the evolutionary lines of horse and human separated, as estimated by paleontologists, we conclude that each chain has on the average suffered an evolutionarily effective mutation every 14.5 million years. We may then use this value to discuss other evolutionary epochs.

The gorilla alpha chain and the human alpha chain differ in two residues, and the gorilla beta chain and human beta chain differ in one; the average, 1.5, indicates that about 11 million years has gone by since the derivation of these chains from their common chain ancestor - that is, that the evolutionary lines leading to the present-day gorillas and present-day human beings separated from one another about 11 million years ago. The estimates made by paleontologists for this epoch range from 10 million to 35 million years.

Another interesting question is that of the biochemical differences between adult human beings and human fetuses. The human fetus manufactures a special kind of hemoglobin, called hemoglobin F. In hemoglobin F the beta chains are abnormal. These abnormal beta chains, which are called gamma chains, differ from adult human beta in 36 of the 146 amino-acid residues. Accordingly we cal-

culate, assuming that there has been a constant rate of evolutionarily effective mutation, that the gamma chains and the beta chains separated from one another about 260 million years ago; that is, at the beginning of the Carboniferous period.

This epoch was, of course, long before human beings had come into existence. Other mammals also have fetal hemoglobins differing from the adult hemoglobin for the species, and we might conclude that the fetal forms of different mammals separated from the adult forms some 260 million years ago, and in a sense constitute a group of species different from the adult group. With respect to hemoglobin, a human fetus resembles a fetal horse more closely than a human adult.

I believe that it will be possible, through the detailed determination of amino-acid sequences of hemoglobin molecules and of other molecules, to obtain much information about the course of the evolutionary process, and to illuminate the question of the origin of species.

Moreover, I believe that the continued study of the molecular structure of the human body and the nature of molecular disease will provide information that will contribute to the control of disease and will significantly diminish the amount of human suffering. Molecular biology and molecular medicine are new fields of science that can be greatly developed for the benefit of mankind.

References

L. Pauling, H. A. Itano, S. J. Singer, and I. C. Wells, "Sickle Cell Anemia, a Molecular Disease," Science 110, 543 (1949).

L. Pauling, "Abnormality of Hemoglobin Molecules in Hereditary Hemolytic Anemias", The Harvey Lectures. XLL, 216, Academic Press Inc., 1955.

E. Zuckerkandl and L. Pauling, "Molecular Disease, Evolution, and Genic Heterogeneity," Horizons in Biochemistry P. 189, Academic Press, Inc., 1962.

V. M. Ingram, "The Hemoglobins in Genetics and Evolution," Columbia University Press, 1963.